

**INFRA-SPECIFIC VARIATION IN *JUNIPERUS DEPPEANA*
AND F. *SPERRYI* IN THE DAVIS MOUNTAINS OF TEXAS:
VARIATION IN LEAF ESSENTIAL OILS AND RANDOM
AMPLIFIED POLYMORPHIC DNAs (RAPDS)**

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ABSTRACT

A recent report of a possible *Cupressus arizonica* growing in the Davis Mtns., prompted field work to collect samples from the furrowed bark tree (Bridge Spring) and compare these with another tree with furrowed bark (Elbow Canyon) as well as typical *Juniperus deppeana* and *Cupressus arizonica*. During the collections, two trees were found that had only juvenile leaves and very elongated terminal whips, so they were included in the analyses of the leaf essential oils and DNA fingerprinting (RAPDs). The trees with furrowed bark and those with elongated terminal whips all had DNA bandings typical of *J. deppeana* in the area, not like *Cupressus arizonica*. Analyses of the leaf essential oils showed both the furrowed bark and elongated terminal whips trees to have oil that was typical of *J. deppeana* and not like the oil of *C. arizonica*. The *J. deppeana* oils contained 17 terpenoids not found in the oil of *C. arizonica*. The leaf oil of *C. arizonica* contained 29 compounds that were not found in the oils of the *J. deppeana* trees. The Bridge Spring tree that has been previously reported as *Cupressus arizonica*, is identified as *Juniperus deppeana* f. *sperryi* with foliage rather erect than drooping. A second tree of *J. d. f. sperryi* was found in Elbow Canyon. The two trees with almost all juvenile leaves and elongated terminal whips are recognized as a new forma, *Juniperus deppeana* f. *elongata* R. P. Adams.

KEY WORDS: *Juniperus deppeana*, *J. d. f. sperryi*, *J. d. f. elongata*, *Cupressus arizonica*, Cupressaceae, terpenes, DNA, RAPDs, systematics, essential oil.

Recently, there has been some confusion concerning the occurrence of *Cupressus arizonica* in the Davis Mtns. of west Texas. Karges and Zach (2001) reported finding a tree of *Cupressus arizonica* on the Nature Conservancy's Davis Mtns. preserve just below Bridge Spring. The specimen was apparently without female cones, so identification was based on leaf morphology. It is very difficult to separate some *Cupressus* and *Juniperus* species using only leaf data. The Bridge Spring juniper has bark exfoliating in interlaced strips, but with quadrangular bark at the very base of the trunk. Subsequently, cpDNA sequences from this specimen were compared with *C. arizonica*, *J. deppeana* and other *Juniperus*, *Calocedrus*, *Chamaecyparis*, and *Thuja* species (Griffith and Bartel, 2002). The cpDNA data (Griffith and Bartel, 2002) showed the putative *C. arizonica* (Karges and Hedges 2480, Karges *s. n.*) from Bridge Spring to form a clade with *J. deppeana*. Although that clade was only supported by a 63 bootstrap value, the clade, including the Bridge Spring tree was nested within other *Juniperus* clades. Karges and Zech (2003) questioned some procedures in the Griffith and Bartel (2002) analysis, such as the lack of use of *J. deppeana* samples from the Davis Mtns., and the citation of Karges *s. n.* specimen with cone scales. Karges and Zech (2003) state that such a specimen did not exist and that only the Karges and Hedges 2408 specimen without cones exists.

The general confusion concerning identifying *J. deppeana* trees with furrowed bark (cf. f. *sperryi*) in the Davis Mtns. led us to re-examine trees reported as f. *sperryi* or allies as well as the Bridge Spring tree. *Juniperus deppeana* f. *sperryi* (Correll) R. P. Adams is documented only by specimens from the type tree on the H. E. Sproul ranch (*O. E. Sperry T870, Adams 352*) but furrowed bark trees are reported to occur in other areas of the Davis Mtns.

MATERIALS AND METHODS

Specimens used in this study: *Juniperus deppeana* var. *deppeana*, Adams 10621-10625, Nature Conservancy's Davis Mtns. Preserve, TX, USA; *J. deppeana* f. *sperryi*, Adams 352, Type tree, Sproul Ranch, Adams 10626, Bridge Spring and Adams 10628, Elbow Canyon, Davis

Mtns., TX, USA; *J. deppeana* f. *elongata*, Adams 10627, 10629, Davis Mtns., TX, USA; *Cupressus arizonica*, Adams 6906, 10650, 10651, cultivated, Waco, TX, USA; Adams 9268, 9269, (ex. Stephanie C. Bartel), Boot Canyon, Chisos Mtns., TX, USA. Voucher specimens are deposited at Baylor University (BAYLU).

Fresh leaves (200 g. fresh wt.) were steam distilled for 2 h using a circulatory Clevenger apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (48h, 100°C) for determination of their oil yields.

The essential oils were analyzed on a HP5971 MSD mass spectrometer, directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2001 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2001), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by TIC.

Sampling for RAPD data -- One gram (fresh weight) of foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by the Qiagen DNeasy mini kit (Qiagen Inc. Valencia CA). The RAPD analyses follow that of Adams and Demeke (1993). Ten-mer primers were purchased from the University of British Colombia (5'-3'): 134, AAC ACA CGA G; 153, GAG TCA CGA G; 184, CAA ACG GCA C; 212, GCT GCG TGA C; 218, CTC AGC CCA G; 239, CTG AAG CGG A; 249, GCA TCT ACC G; 250, CGA CAG TCC C; 268, AGG CCG CTT A; 327, ATA CGG CGT C; 338 CTC TGG CGG T; 346, TAG GCG AAC G; 347, TTG CTT GGC G; 413, GAG GCG GCG A; 478, CGA GCT GGT C.

PCR was performed in a volume of 15 µl containing 50 mM KCl, 10 mM Tris-HCl (pH 9), 2.0 mM MgCl₂, 0.01% gelatin and 0.1%

Triton X-100, 0.2 mM of each dNTPs, 0.36 μ M primers, 0.3 ng genomic DNA, 15 ng BSA and 0.6 unit of Taq DNA polymerase (Promega). A control PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). The thermal cycle was: 94°C (1.5 min) for initial strand separation, then 40 cycles of 40°C (2 min), 72°C (2 min), 91°C (1 min). Two additional steps were used: 40°C (2 min) and 72°C (5 min) for final extension.

DNA bands that occurred once were not scored. It should be noted that these bands contain very useful information for the study of genetic variance and individual variation, but are merely "noise" in the present taxonomic study. Bands were scored in 4 classes: very bright (=6); medium bright (=5), faint (=4) and absent (=0). See Adams and Demeke (1993) for details on electrophoresis and RAPD band scoring.

Similarity measures were computed using absolute character state differences (Manhattan metric), divided by the maximum observed value for that character over all taxa (= Gower metric, Gower, 1971; Adams, 1975).

RESULTS AND DISCUSSION

Two trees were discovered that had elongated terminal whips and mostly juvenile leaves (decurrent) on otherwise mature trees. The long terminal whips (15- 30 cm) give the trees a weeping appearance. One of these trees (*Adams, 10627*, in grassland, 1845 m, on the n. side of Tex. 118, 2.6 mi. west of the w. entrance to Lawrence E. Wood Madera Ck. roadside park) has been reported to RPA by Tom Van Devender (pers. comm.) as a possible *J. d. f. sperryi* tree. The second tree with weeping foliage, elongated whips, and only juvenile leaves at the summit of Brown Mtn., 2190 m, (*Adams 10629*), Davis Mtns., was shown to RPA by John Karges. These trees were found to have oil and DNA fingerprints like typical *J. deppeana* from the Davis Mtns. Thus, it appears that they differ in only a few genes that are expressed occasionally among otherwise typical *J. deppeana* plants. This elongated whip form is confused with *J. d. f. sperryi* by field workers.

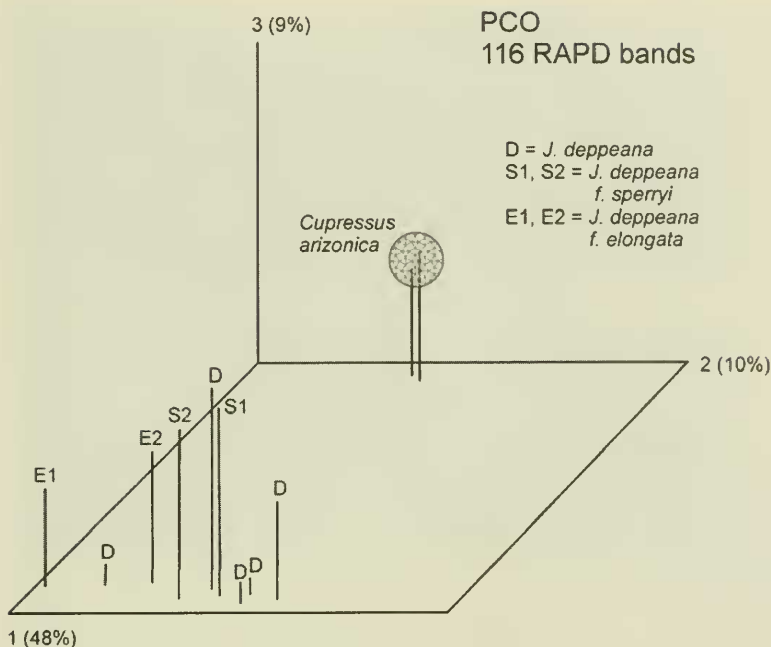


Figure 1. Principal Coordinates Ordination (PCO) based on 116 RAPD bands. The Bridge Spring tree (S1) and the other interlaced bark tree from Elbow Canyon (S2) are interspersed with typical *J. deppeana* along with the two trees of *J. d. f. elongata*.

To recognize this variation and prevent future confusion, a new forma of *J. deppeana* is proposed:

Juniperus deppeana Steud. f. *elongata* R. Adams, *forma nov.* TYPE: Texas, USA, on Tex. 118, 4.2 km west of w. entrance to Lawrence E. Wood Madera Ck. park, 1845 m, Lat. 30 43.437' N; Long. 104 08.255' W, 11 March 2005, R. P. Adams 10627 (HOLOTYPE: BAYLU, ISOTYPE: SRSC)

J. deppeanae typicae similis sed differt foliis ramulorum elongatorum plerumque decurrentibus juvenalibus; ramuli demissi.

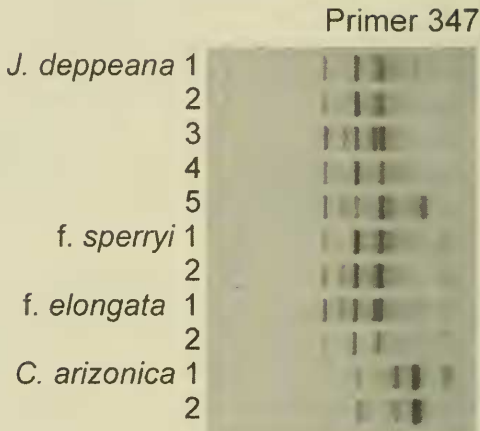


Figure 2. Agarose gel showing the banding using primer 347 for *Juniperus deppeana* and *Cupressus arizonica*. The *f. sperryi* 1 sample is the tree from Bridge Spring and *f. sperryi* 2 is the tree from Elbow Canyon. Both are clearly a part of typical *J. deppeana* (1-5 above).

Similar to typical *J. deppeana* but different in the leaves of the elongated branchlets mostly decurrent and juvenile; branchlets drooping.

Other specimen examined: U.S.A.: Texas, Davis Mtns., Brown Mtn. 2190 m (summit), *R. P. Adams 10629* (BAYLU, SRSC).

Analysis of the leaf essential oils of the plants in this study is presented in table 1. Notice the Bridge Spring tree (S2) and the Elbow Canyon tree (S3) have oils that are like *J. deppeana* from the Davis Mtns. and *J. deppeana f. elongata* (E1, E2), but quite different from *Cupressus arizonica* oil. Seventeen compounds were found in the *J. deppeana* plants (including *f. sperryi* and *f. elongata*) that were not found in *C. arizonica* (table 1). Conversely, 29 compounds were found only in *C. arizonica*. Umbellulone was 11.2% in *C. arizonica*, but absent in all the *J. deppeana* samples. The diterpenes were noticeably different in *C. arizonica*: *cis-14-nor-muulol-5-en-4-one*,

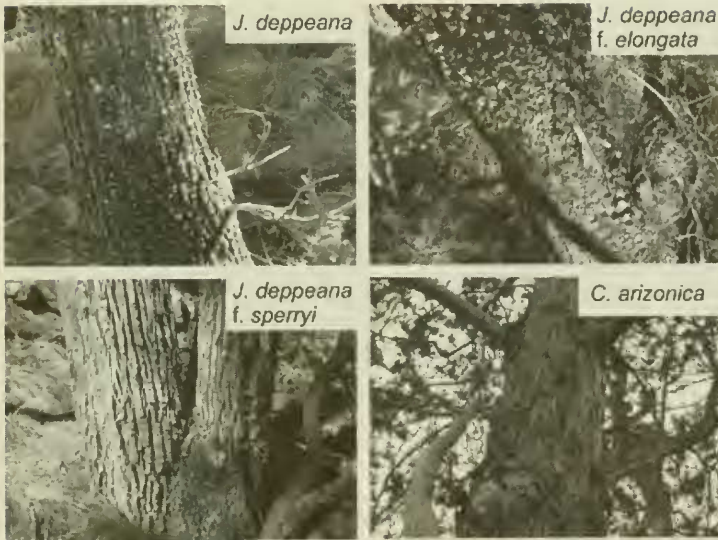


Figure 3. Bark of *J. deppeana*, *J. deppeana* f. *elongata*, *J. deppeana* f. *sperryi* (Bridge Spring tree) and *Cupressus arizonica*. Note the quadrangular bark at the base of the Bridge Spring tree.

isopimara-9(11),15-diene, isohibaene, sandaracopimara-8(14),15-diene, isophyllocladene, phyllocladene, abieta-8,12-diene, nezukol, phyllocladanol, semperviol and trans-totarol. Of the 132 compounds quantitated within *J. deppeana* - *C. arizonica* (table 1), 46 were found only in *Juniperus* (17) or in *Cupressus* (29). It is clear that the leaf oils of *J. d.* f. *sperryi* and f. *elongata* are quite similar to the oil of *J. deppeana* from the same area.

Principal coordinate analysis (PCO) of 116 RAPD bands extracted eigenroots accounting for 48.3, 9.5, 8.7, 7.7, 5.8% of the variance among the samples. These eigenroots appear to asymptote after the second or third root, implying lack of significance for subsequent eigenroots. PCO of the first three axis reveals that the first eigenroot (48%) separates *C. arizonica* from *Juniperus* samples (Fig. 1). The individuals of *J. deppeana*, *J. d.* f. *sperryi* (S1 is the Bridge Spring individual), and *J. d.* f. *elongata* are all interspersed (Fig. 1).



Figure 4. Habit and foliage of *J. deppeana* f. *elongata*, Holotype (left) and Brown Mtn. (right) trees.

Figure 2 shows a gel photo for primer 347. The major bands present in *Juniperus* samples are absent in *Cupressus* samples and the major bands in *Cupressus* are absent in *Juniperus*. Although there are some polymorphisms in the *J. deppeana* samples, they are clearly part of a defined group (as seen in the 116 RAPD based PCO, Fig. 1).

The bark of *J. deppeana* varies (Adams, 2004) from quadrangular (in var. *deppeana* and var. *robusta*) to exfoliating in narrow, often interlaced strips (in var. *patoniana* and f. *sperryi*). A comparison of bark variation in the Davis Mtns. is shown in figure 3 along with bark from *C. arizonica*. The bark of the *J. d.* f. *sperryi*, Bridge Spring tree exfoliates in interlaced strips on the upper trunk, but in quadrangular plates at the base of the trunk (Fig. 3). Notice (Fig. 3) that the bark of the type tree of *J. d.* f. *elongata* has an unusual, thin quadrangular scaly bark. This might be the result of the tree being genetically fixed in the juvenile leaf form. The presence of only juvenile leaves on adult juniper trees is rare, but has been reported (Adams, 2004) in many *Juniperus* species. However, some juniper species are characterized by having only or mostly juvenile leaves (*J. chinensis*) on adult trees.

The general habit of the type tree of *J. deppeana* f. *elongata* (Adams 10627) and a close up of the elongated terminal whips on another tree of *J. d.* f. *elongata*, at the summit of Brown Mtn., are shown in figure 4. At present, these are the only two known trees of the new forma, but it is likely that additional trees will be discovered.

We now know of three trees of *J. d.* var. *sperryi* in the Davis Mtns., but again, additional trees will likely be discovered.

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Table 1. Comparisons of the per cent total oil for leaf essential oils for *J. deppeana* (DP), *J. deppeana* f. *sperryi* (S1, Adams 352, Sproul Ranch; S2, Adams 10626, Bridge Spring; S3, Adams 10628, Elbow Canyon), *J. deppeana* f. *elongata*, (E1, Adams 10627; E2, Adams 10628) and *Cupressus arizonica* (CAz, average of three tree oils, Adams 6906, 10650, 10651). Compounds are in boldface that separate the taxa.

K1	Compound	DP	S1	S2	S3	E1	E2	CAz
926	tricyclene	0.4	0.1	t	0.9	0.1	t	t
931	α -thujene	0.3	0.3	t	0.6	0.2	0.3	0.5
939	α -pinene	8.2	12.7	3.8	3.8	5.1	9.2	7.2
953	α -fenchene	t	-	-	-	-	-	t
953	camphene	0.7	0.3	0.2	1.1	0.3	0.7	0.2
957	thuja-2,4(10)-diene 0.1	t	-	-	t	0.3	-	-
967	verbenene	0.1	1.0	0.2	0.1	t	-	-
976	sabinene	5.2	5.2	2.4	8.0	5.2	3.2	2.6
980	β -pinene	0.7	2.0	0.3	0.7	0.5	0.3	0.3
991	myrcene	2.3	2.9	2.1	3.7	2.6	1.6	2.0
1001	δ-2-carene	0.1	t	t	0.2	0.2	3.4	-
1005	α -phellandrene	1.0	0.7	1.2	2.1	2.0	0.8	t
1011	δ -3-carene	4.9	t	0.3	2.4	-	4.0	0.3
1018	α -terpinene	0.7	1.2	0.7	1.2	1.0	0.5	0.8
1026	p-cymene	1.4	3.8	0.9	2.1	1.3	1.8	0.7
1031	limonene	1.0	t	1.0	t	0.1	2.9	3.0
1031	β -phellandrene	8.0	7.0	9.5	13.7	10.9	5.7	3.2
1050	(E)- β -ocimene	0.2	0.2	0.2	0.2	0.2	0.1	-
1062	γ -terpinene	1.2	2.8	2.1	2.2	1.6	0.8	1.2
1068	cis-sabinene hydrate	0.7	3.4	2.1	1.5	0.5	0.1	0.5
1074	trans-linalool oxide	0.1	-	-	-	-	0.3	-
1082	m-cymenene	0.1	-	-	-	-	0.4	-
1088	terpinolene	1.7	1.1	1.6	1.7	1.5	1.6	1.1
1091	6,7-epoxymyrcene	0.1	-	-	-	-	-	-
1096	96, 109, 152, terpene alcohol	-	1.1	-	-	-	-	-
1097	trans-sabinene hydrate	-	-	-	-	-	-	0.3
1098	linalool	4.1	2.9	5.5	1.7	5.7	2.8	0.4
1121	cis-p-menth-2-en-1-ol	1.3	1.7	1.6	1.2	1.7	1.4	0.3
1125	α -campholenal	0.5	0.2	0.3	t	0.3	1.3	t
1134	cis-limonene oxide	0.2	-	-	-	-	0.8	-
1140	trans-p-menth-2-en-1-ol	1.1	1.3	0.8	-	t	1.9	0.4
1143	camphor	14.1	7.6	26.5	19.9	18.7	3.1	1.2
1148	camphene hydrate	0.9	0.6	1.3	0.9	0.9	0.4	t
1156	sabina ketone	0.4	0.3	-	-	0.4	0.6	-
1161	92, 79, 94, 152, terpene alcohol	-	-	-	-	-	1.1	-
1165	borneol	-	0.3	-	-	-	-	-
1168	umbellulone(=3-thujen-2-one)	-	-	-	-	-	-	11.2
1170	p-mentha-1,5-dien-8-ol	0.9	0.2	0.5	0.7	0.8	2.2	-
1173	cis-pinocamphone	0.2	0.6	-	-	-	0.1	-

1536	italicene ether	-	-	-	-	-	-	1.1
1540	α -copaen-11-ol	-	-	-	-	-	0.8	-
1542	α -calacorene	-	0.1	-	-	-	t	-
1550	cis-muurolo-5-en-4- β -ol	-	-	-	-	-	-	2.6
1549	elemol	0.5	2.7	0.4	0.3	0.7	t	-
1559	cis-muurolo-5-en-4- α -ol	-	-	-	-	-	-	3.0
1574	germacrene D-4-ol	-	-	-	0.2	-	-	-
1581	caryophyllene oxide	0.5	0.4	0.1	0.3	0.3	0.3	0.2
1596	cedrol	0.3	t	t	-	0.3	0.7	1.2
1606	humulene epoxide II	0.4	0.2	0.7	0.3	0.4	0.2	0.3
1618	1,10-di-epi-cubenol	-	-	-	-	-	-	0.3
1627	1-epi-cubenol	1.7	0.9	1.5	1.2	1.6	1.1	-
1630	α -acoreno	-	-	-	-	-	-	3.4
1637	β -acoreno	-	-	-	-	-	-	0.6
1640	epi- α -cadinol	0.2	0.1	0.4	0.4	0.2	0.1	0.3
1640	epi- α -muurolo	0.3	0.2	0.4	0.4	0.3	0.1	-
1645	α -muurolo (= torreyol)	0.2	t	0.1	t	t	t	-
1649	β -eudesmol	0.2	0.4	t	t	0.2	t	-
1652	α -eudesmol	0.1	-	-	-	0.1	-	-
1653	α -cadinol	0.2	0.3	0.7	0.4	0.1	t	1.1
1674	cadalene	0.1	-	-	-	-	t	0.3
1689	cis-14-nor-muurolo-5-en-4-one	-	-	-	-	-	-	2.6
1789	8- α -acetoxyelemol	0.1	t	1.5	0.3	0.3	t	-
1906	isopimara-9(11),15-diene	-	-	-	-	-	-	0.1
1933	isohibaene	-	-	-	-	-	-	0.2
1960	sandaracopimara-8(14),15-diene	-	-	-	-	-	-	0.2
1998	epi-13-manoyl oxide	1.2	0.7	2.3	t	1.5	2.5	-
1966	isophyllocladene(=kaur-15-ene)	-	-	-	-	-	-	0.1
2017	phyllocladene	-	-	-	-	-	-	0.1
2017	manoyl oxide	t	t	t	-	t	t	-
2022	abieta-8,12-diene	-	-	-	-	-	-	0.1
2054	abietatriene	0.1	0.2	t	-	t	t	0.2
2080	abietadiene	0.2	0.1	0.1	0.1	0.4	0.2	1.9
2133	nezukol	-	-	-	-	-	-	3.7
2210	phyllocladanol	-	-	-	-	-	-	3.9
2283	semperviol	-	-	-	-	-	-	0.3
2288	4-epi-abietal	t	t	-	-	-	-	-
2314	trans-totarol	-	-	-	-	-	-	0.3
2302	abieta-7,13-dien-3-one	0.6	0.1	0.4	0.5	0.7	0.7	-
2325	trans-ferruginol	t	t	t	-	t	-	t

KI = Kovat's Index on DB-5(= SE54) column. *Tentatively identified.

Compositional values less than 0.1% are denoted as traces (t).

Unidentified components less than 0.5% are not reported.